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BOTULISM FROM EATING CANNED RIPE OLIVES.[1]

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INTRODUCTION.

Cases of poisoning now recognized as botulism have been reported from time to time since as early as 1735; and from this time to the present, outbreaks of botulism have been recorded with increasing frequency. No historical review or survey of the now fairly extensive literature is here attempted. Those interested in this feature of the matter are referred to Dickson's Monograph, No. 8, Rockefeller Institute for Medical Research.

From 1910 to 1916, inclusive, 3,916 deaths from food poisoning were recorded in the registration area of the United States. There is, thus, for this period, an estimate of 874 deaths annually among the population of the United States due to food poisoning. Just what proportion of these deaths is due to botulism is, of course, unknown; but when the difficulty of diagnosis is remembered, together with the frequent report of deaths from "ptomaine," it is likely that botulism in America is more common than the reports would indicate.

The *Bacillus botulinus* was first isolated by von Ermengem in 1894, from ham, and his observations have been confirmed by various writers. (See Dickson's Monograph.) Further significance has recently been given to *Bacillus botulinus* in this country by Graham and Brueckner, who isolated an organism from ensilage and from oat hay which had caused outbreaks of forage poisoning in horses and mules. This organism seems to be a strain of *Bacillus botulinus*.[2] Forage poisoning is said to have caused in 1912 the death of 20,000 mules in Kansas, Missouri, and Nebraska, and sporadic outbreaks have occurred from time to time in Kentucky, Illinois, and other States.[3]

Bacillus botulinus has been found in nature in oat hay, ensilage, and in the intestinal contents of a normal pig, by Kemper and Pollack.[4] In Europe, botulism has been most frequent in Germany, and

[1] From the State department of health, Columbus, Ohio.
[2] Graham and Brueckner, Jour. Bacteriology, January, 1919.
[3] Idem.
[4] Deutsch. Med. Woch. 1897, XXIII, 505.

usually has followed the use of poorly cooked meats—sausage, ham, etc. The well known outbreak at Dernstadt reported by Landmann is an exception, having been caused by canned white beans. In America, however, botulism has most often been associated with the use of home-canned fruits and vegetables. It is of interest to note that of 64 cases recorded by Dickson in the United States during the past 25 years, 54 occurred in California. The outbreak described in this article was due to eating California packed fruit. This outbreak is contrary to the experience of Weinzirl [1] in that it was caused by commercial canned goods. This is especially disturbing, as one can hardly fail to appreciate the possibility of many jars being infected at the same pack, and of the organism being sent broadcast over the country with its attending hazards.[2] It would appear, moreover, that olives are especially dangerous, since they are usually served without cooking, a process which destroys the toxin of *Bacillus botulinus*.

Canned pears, string beans, white beans, asparagus, peas, corn, apricots, spinach, artichokes, and peaches have been known to either produce cases of botulism or to have permitted the growth of *Bacillus botulinus* and toxin development experimentally.

STUDY OF OUTBREAK FROM EATING RIPE OLIVES.

The outbreak of poisoning here considered developed in a group of people who were in attendance at a banquet held on the evening of August 23, 1919, at a country club near Canton, Ohio. There were present at this banquet about 200 people from Canton and the surrounding towns.

Following the dinner 14 cases of poisoning occurred—11 among guests and 3 among the employees at the club. Five guests and 2 employees died. The guests who became ill were all members of a party given by Mrs. I. W. G., of Sebring, Ohio, and had been served at a separate table which shall hereafter be designated as the Sebring table. The two waiters who attended this table and the chef were also affected.

The Menu.

The following foods were served at the banquet:

Canteloupe	Green olives, celery, and pickles
Turkey	Rolls
Turkey stuffing	Butter
Tomatoes and mayonnaise	Ice cream
Crackers	Cake
Scalloped corn and pimentoes	Water
Browned potatoes	Coffee.

[1] Jour. Medical Research, January, 1919.

[2] Since this paper was written an outbreak of poisoning near Detroit, Mich., has come to our attention. In this outbreak there were 5 deaths attributed to botulism from the eating of ripe olives of the same brand found responsible for the poisoning herein described.

The Sebring table was served, in addition to the above, with ripe olives, chocolate candy, Newport creams, and candied almonds, all of which were furnished by the hostess. The green olives, celery, and pickles were not served at this table.

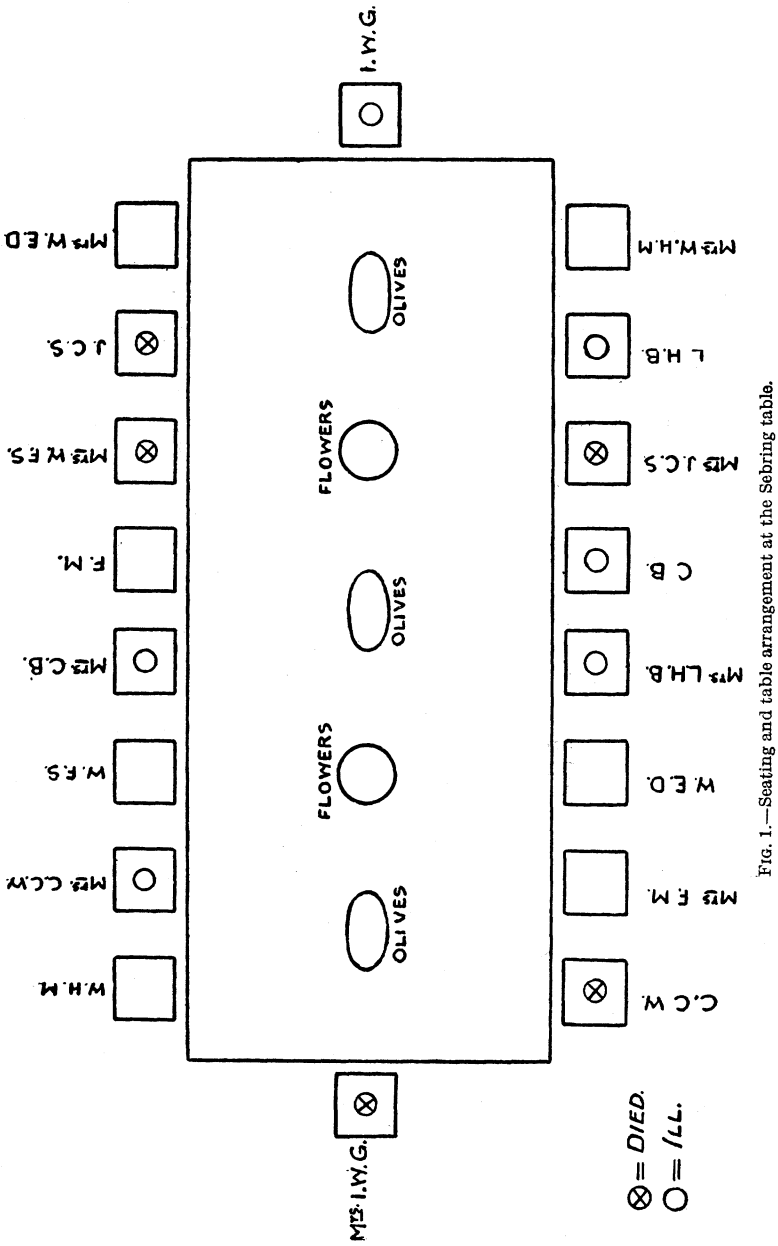

FIG. 1.—Seating and table arrangement at the Sebring table.

Soft drinks were dispensed at the grill downstairs, and a few persons had partaken of alcoholic beverages from their individual stocks.

The symptoms of those affected were so similar as to point to a common cause; and since those affected had had no other meetings, food, or drinks in common, and since no other foods were served, it would seem that the toxic substance was something in the above-mentioned menu, and something restricted to the Sebring table.

The Epidemiological Investigation.

The investigation was begun on August 29, seven days after the banquet, and after 6 of the cases had terminated fatally. Each member of the Sebring party and each of the club employees, excepting the fatal cases, was interviewed to ascertain whether or not he had partaken of the various articles served at the banquet.

Some 15 people from various parties, other than the party at the Sebring table, were interviewed, and the bill of fare as served at their various tables was found to be identical with that served at the Sebring table, excepting that green olives, celery, and pickles were served in place of the ripe olives, candy, and nuts, which were furnished especially by Mrs. I. W. G. for her guests. No illness occurred among the banqueters from the other tables.

In the attempt to learn what foods people had or had not eaten at the banquet, only definite information was recorded. All such answers as: "I think I ate it, but not certain," "I like it and probably did," etc., were recorded as doubtful. In the case of the deceased, the only evidence accepted was their own ante mortem statements, or the statements of others at the table who saw them eat this or that article. Evidently the fact that they were not observed to have eaten any particular substance could not be accepted as evidence that they had not done so.

The scene of the banquet was inspected, and the manager and employees were interviewed as to the source of supply, mode of preparation, and serving of the various articles used at the dinner. The epidemiological evidence and other facts which seem of importance will be considered with reference to each article of the menu. (See Table I.)

Cantaloupe.—The cantaloupes were the choice ripe fruit selected from 9 cases of melons. Each guest received one-half a melon, and the melons served at the Sebring table were similar to those served to all. The remainder of the 9 cases of melons was eaten later by other persons, and no ill effects followed. Moreover, two persons who were poisoned had not partaken of their melon. It would seem, therefore, that the melons may be excluded from further consideration.

Turkey.—The turkeys were cold-storage fowls. Twelve in all were purchased for this dinner, and 9½ were served. The turkeys were "drawn" on August 23, and cooked on the afternoon of that

day. They were carved by two people working at the same table but on separate birds, and the carved portions were placed upon a single large "hot plate" until served. The waiters filed in and received the turkey for their respective tables whenever the dinner at their particular table had reached the meat course. Guests and waiters agree that the Sebring table was neither early nor late, but was served at a time when many tables were being served. Each plate was supplied with light and dark meat and with dressing. Among those interviewed who ate turkey, all agree that no rare meat was served. With the exception of two persons, both of whom were but slightly ill and who thought the meat "a little slimy," all agreed that the turkey was "excellent," saying that they had "never eaten better," "it was tiptop," etc. Since about 200 people were served from 9½ birds, one bird should have supplied about 20 people. Assuming turkey to be the cause of the illness, it would seem reasonable, from the number affected, to conclude that the toxic substance was confined to one, or a portion of one, bird; but when the manner of serving is considered, it seems improbable that the toxic portion should have been delivered to one table only, and still more improbable if we assume the poison to have been from portions of several fowls.

Table I.—*Food eaten by members of the Sebring party, and its effect.*

Club help and guests of the Sebring table.	Effect.		Food eaten.																		
				Turkey.																	
	Ill.	Died.	Melon.	Light.	Dark.	Turkey stuffing.	Ripe olives.	Tomatoes and mayonnaise.	Corn and pimentoes.	Browned potatoes.	Crackers.	Rolls.	Butter.	Ice cream.	Cake.	Chocolate candy.	Newport creams.	Water.	Almonds.	Coffee.	Soft drinks.
C. C. W. ♂	+	+	+	+	+	+	+	+	?	?	?	+	+	?	+	+	+	+	+	+	?
I. W. G. ♀	+	+	?	+	+	+	+	?	?	?	?	?	?	?	?	?	?	?	?	+	?
J. C. S. ♂	+	+	+	+	+	+	+	?	?	?	?	+	+	+	+	?	+	+	+	?	?
J. C. S. ♀	+	+	?	?	?	?	+	?	?	?	?	?	?	+	+	?	?	?	+	?	?
W. F. S. ♀	+	+	+	+	+	+	+	?	?	?	?	?	?	?	?	?	?	?	?	?	?
F. Mc. A. ♂	+	+	0	0	0	0	+	?	?	?	?	?	?	?	?	?	?	?	?	?	?
R. J. ♂	+	+	?	?	?	?	+	?	?	?	?	?	?	?	?	+	+	?	?	?	?
C. B. ♀	+	0	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	0	0
L. H. B. ♀	+	0	+	+	+	+	+	+	0	?	+	+	+	+	+	?	+	+	0	+	0
L. H. B. ♂	+	0	+	+	+	0	+	0	?	?	?	+	+	0	0	+	+	+	+	0	0
C. O. ♂	+	0	0	+	+	+	+	0	+	?	0	0	0	0	0	0	0	+	0	0	+
I. W. G. ♂	+	0	+	+	0	+	+	?	?	+	?	0	0	+	+	0	+	+	+	?	+
C. C. W. ♀	+	0	+	0	0	+	+	+	+	0	+	+	+	+	+	0	+	+	+	0	0
C. B. ♂	+	0	+	+	+	+	+	0	+	+	0	+	+	+	+	+	+	+	+	+	0
W. H. M. ♀	0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0
W. E. D. ♀	0	0	+	+	+	+	+	+	+	?	+	+	+	+	+	+	+	+	+	+	0
W. E. D. ♂	0	0	+	+	+	+	+	0	0	+	+	+	+	+	+	+	0	+	+	0	0
W. H. M. ♂	0	0	+	+	+	+	0	+	+	+	+	+	+	+	+	0	+	+	+	+	0
W. F. S. ♂	0	0	+	+	?	+	0	+	0	+	+	+	+	+	+	0	+	+	+	+	0
F. M. ♀	0	0	+	+	0	+	0	0	+	0	+	+	+	+	+	+	+	+	?	+	0
F. M. ♂	0	0	+	+	0	+	0	+	+	+	+	+	+	+	+	0	0	+	+	0	0
J. A. ♀	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	0	0	+
J. A. ♂	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	0	0	+
J. S. ♂	0	0	+	+	+	+	0	?	+	?	0	+	+	0	0	+	+	+	+	+	0
J. G. ♂	0	0	0	+	+	+	0	0	0	0	0	+	+	+	?	+	+	+	+	0	+
J. F. ♂	0	0	0	+	+	+	0	+	+	+	0	0	0	+	+	0	0	+	?	+	0
S. R. A. ♂	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0
A. C. L. ♂	0	0	0	0	0	0	0	+	+	?	?	+	+	+	+	0	0	+	?	+	0
A. M. ♀	0	0	0	+	+	+	0	0	+	?	+	+	+	+	+	0	0	+	0	+	0
J. C. ♂	0	0	0	+	+	+	0	?	+	?	?	?	?	+	+	0	0	+	0	?	0
C. R. ♂	0	0	0	+	+	+	0	+	0	?	?	+	+	0	0	0	0	+	0	0	0
H. B. S. ♂	0	0	0	+	+	+	0	+	+	+	?	+	+	0	0	0	0	+	0	+	0

The banqueters were quite definite in their recollections as to whether or not they had eaten their turkey, their attention having been early directed to turkey as a possible causative agent by the published statements of several physicians, who gave it as their opinion that turkey was the cause of the poisoning due to infection with *Bacillus botulinus*. This theory seems improbable, however, in view of the fact that the toxin of *botulinus* is easily destroyed by heat, and all evidence points to the turkey having been well cooked. Furthermore, it was not cold from time of cooking to serving, which at most was a matter of a few hours or minutes.

Among the 14 people showing symptoms, 9 ate both white and dark meat, 1 ate only white meat, and there is doubt in the case of 2 who died. The chef stated before death that he ate no turkey, and the kitchen help at the club testify to this. One other case, a mild one, had eaten no turkey. All of the 7 unaffected diners at the Sebring table ate turkey. Among the 9 unaffected employees at the club, 7 ate turkey. It would seem difficult, therefore, to explain the poisoning on the assumption that it was caused by the turkey.

Turkey stuffing.—There appears little need to consider the stuffing independently of the turkey; all but one of the affected who ate turkey also ate dressing, and one who ate no turkey ate of the stuffing. One of those who became ill ate neither turkey nor stuffing.

Tomatoes and mayonnaise.—The tomatoes were grown in the club gardens. They were picked on the morning of August 23, the day of the dinner, and were sliced directly into the serving plates about two hours before serving. The mayonnaise was made at the club on August 23, and enough was made in one mixing to serve all. Three of those taken ill ate neither tomatoes nor mayonnaise; 4 of the ill ate both; in 7 there is doubt. Six of the 7 who were not poisoned at the Sebring table ate tomatoes and mayonnaise.

Corn and pimentoes.—The corn was grown in the club garden and was picked and cooked on the morning of the dinner. It was cut from the cob and was prepared by mixing it with two cans of pimentoes. The corn and pimentoes were prepared in one pan and at one mixing. It seems apparent, therefore, that any poison in this dish would not have been localized at one table. Four of the 14 who were ill remembered eating their portions; 1 ate none; in 9 cases there is doubt. Among the nonaffected at the Sebring table 5 ate corn and pimentoes, while 2 ate none.

Browned potatoes.—The potatoes served were all from the same source, and were prepared in the same kettle and browned in the same pan. Three of those who became ill ate potatoes, 1 ate none, while in the other 10 there is doubt. Potatoes were eaten by 5 of the 7 nonaffected at this table. It seems hardly possible to explain the limitation of the poisoning to one group on the assumption that the cause was in the potatoes.

Crackers.—One brand of crackers was served to all. Two of those who became ill ate none; 3 of the ill ate them, and in the case of the other 9 there is doubt. Among the 7 nonaffected diners at the Sebring table all ate crackers. It would seem that crackers need not be considerded further in connection with the poisoning.

Rolls.—Rolls from the same source were served to all tables. Two of the ill ate none of them, while 13 of the 16 nonaffected employees and diners of the ill-fated table ate them.

Butter.—The butter, in pound packages, was all purchased from one source. Each pound used was divided by a machine into 33 squares for individual serving. It is apparent that a pound of butter, if it contained a toxic substance, should have affected more than 14 persons; and, moreover, it is highly improbable that the whole of any one pound could have reached one table, as the butter dishes were prepared some time before they were served and were taken at random by the waiters. Besides, two persons were ill who ate no butter.

Ice cream.—The ice cream for the 200 guests was served from two 5-gallon cans, about half of each can being used. It is apparent, therefore, that if a freezer of infected ice cream was the source of the trouble, more people should have suffered. The remainder of the two freezers of ice cream which was not eaten at this supper was used at other times and no illness followed. Moreover, 2 who were ill had eaten no ice cream.

Soft drinks.—Sodas, lemonade, etc., were dispensed from the grill, but these drinks were not generally indulged in. Seven of those taken ill drank no soft drinks; in the remaining 7 there is doubt.

Water.—The water served at all the tables was from a single source and was drunk by practically all persons present. There is no evidence pointing toward the water as the vehicle of the poison.

Alcoholic drinks.—No alcoholic drinks were sold at the club, and none was served. There were a few who had drinks from private stocks. Four who were ill, and one at the Sebring table, not ill, partook of alcoholic beverages from private stocks. The remaining 8 who were ill drank none.

Green olives, celery, and pickles.—Green olives, celery, and pickles were served to all diners other than those at the Sebring table. None who partook of these relishes became ill, and of the ill none had eaten of them.

Chocolate candy.—Chocolate candy was furnished especially to the Sebring table by the hostess. Three of the ill ate none of it; 5 ate it; there is doubt in the cases of the remaining 6. Seven who were not ill ate of this candy. Evidently the candy may be eliminated.

Newport creams.—Newport creams were also especially furnished to the Sebring table. Of the ill, 1 had not tasted this candy, 9 had

eaten it, while there is doubt in case of the remaining 4. Eleven people ate freely of it and were not ill.

Candied almonds.—Candied almonds were served only to the Sebring table. Among the 14 affected people, 8 had eaten of the nuts, 4 are doubtful, while 2 who were ill ate none. Among the unaffected, 9 had eaten of the nuts.

Ripe olives.—Ripe olives were also furnished especially for the guests of the Sebring table. During the course of the dinner various diners who tasted the olives observed something peculiar in their taste, odor, or consistency, all of which qualities received more or less comment during and following the dinner. Various members of the party in describing the olives used such expressions as "smelled like limburger," "bit the tongue," "seemed to pucker the mouth," "stuck to the tongue," "not fit to eat," "soft," etc. When certain of the diners developed symptoms, the suspicion by various members of the party that the olives might be the cause prompted them to refresh their memories as to whether or not they had eaten of them.

Of the 14 persons who were ill, all ate olives. Three others who tasted of them used the expressions "just bit into one," "took a small bite," "swallowed not over a third or a half." None of these 3 showed any symptoms which could be definitely identified as similar to those of the above-mentioned 14 definite cases. One, however, states that she felt badly on the day following the banquet, and had symptoms of an indefinite gastrointestinal attack to which she is subject. It is impossible to state whether poison from the dinner may or may not have been a causative factor in these indefinite symptoms.

When the dead are considered it is found in a general way that those died first who ate the most olives. Among those who were ill but recovered those who suffered the severest attacks ate more olives than those who were less severely attacked.

Those who ate olives and were not definitely affected ate the least of all. (See Tables II and III.) The average number of olives eaten by those who died is between 2.5 and 3.5; by those definitely ill but who recovered, 1; by those unaffected, perhaps one-third.

TABLE II.—*Fatal cases—Relation of time elapsed between dinner and death to the number of olives eaten.*

Patient.	Hours elapsed between dinner and death.	Number of olives eaten.
R. J.	54.0	5 or 6
Mrs. I. W. G.	55.5	3
C. C. W.	59.5	4 or 5
J. C. S.	69.0	4 or 5
F. McA.	75.0	2
Mrs. J. C. S.	86.5	1
Mrs. W. F. S.	174.5	0.5
Total		20 to 23

TABLE III.—*Nonfatal cases—Relation of severity of illness to the number of olives eaten.*

Patient.	Order of severity.	Number of olives eaten.
C. O.	1	2
Mrs. L. H. B.	2	1
Mrs. C. B.	3	0.5
L. H. B.	4	0.5
I. W. G.	5	1
Mrs. C. C. W.	6	1 bite.
C. B.	7	1 bite.
Mrs. W. H. M.	Doubtful symptoms	1 bite.
Mrs. W. E. D.	No symptoms	1 bite.
W. E. D.	No symptoms	1 bite.
Total number of olives eaten of.		11

Suspicion is further cast upon the olives by the fact that, although they were in a vacuum-sealed glass jar, something had occurred to destroy the vacuum in the jar; for, in opening it, the lid is said to have come off easily without having been punctured and without the use of instruments. The lid was lost before it was known that any interest might be attached to it. The recovered glass jar was not cracked or defective in any way.

The waiter who received the jar from I. W. G. opened it immediately and placed the olives in three table dishes. The olives placed in two of these he washed under the tap and drained through his fingers, while the olives in the third dish were unwashed. This may possibly aid in explaining the fact that one person, for instance, died from eating one-half an olive, while another recovered after eating two olives. Certainly the washing would remove some poison. Furthermore, it may be that a firm olive with unbroken skin would contain less toxic material than a riper one or one with a broken skin, and, moreover, we know nothing about individual susceptibility or the influence of other articles of food or drink on the effect of the poison. In this connection it is interesting to note that the waiter who ate two olives and recovered drank considerable whisky and other alcoholic drinks both before and after eating the olives, and one guest who ate one olive, and had a few symptoms afterwards, also drank whisky following the dinner.

A bottle of olives of the same size and brand as those used at the dinner of August 23 was found to contain 43 olives. The number said to have been eaten plus the 6 olives recovered amounts to from 37 to 40. It is probable, therefore, that some ate more than our information would indicate. This does not seem remarkable, since the numbers are apt to be less definitely remembered after 3 or 4 are eaten.

The occurrence of poisoning at the Sebring table can be accounted for only by the ripe olives served at this table.

Among the waiters at the club there is a custom of collecting the delicacies after the diners have finished, and the two waiters poisoned

did so collect the left-over olives and ate some of them. Later, waiter C. O. carried the olives to the chef with the request that he "Try one of these damn things, they don't taste right to me." The chef ate two and later died.

Epidemiological Summary.

1. The ripe olives were known to have had a peculiar taste and odor, and in the light of the epidemiological data and circumstances under which the poisoning occurred, it does not seem possible to hold any other article of the menu to be the vehicle of the poison.

2. The limitation of the poison to the diners at the Sebring table, to the waiters of this table, and to the chef, is explained by the theory that the ripe olives were the poisoning agent.

3. Fourteen of the 17 who ate or tasted of the ripe olives were definitely ill.

4. None were ill who did not eat ripe olives.

5. The severity of the illness in each case was, in general, proportionate to the number of ripe olives eaten.

6. The factors that some of the olives were washed before they were eaten while some were not, of our ignorance of the relative toxicity of different olives, of the effects of other articles of food or drink on the poisonous substance, and of individual immunity or susceptibility, together with numerous other factors of unknown effect, would seem to furnish various possibilities for explaining why some recovered after eating more ripe olives than others did who died.

Epidemiological Conclusion.

The poison which caused the death of the 7 people and the illness of 7 others under the circumstances described, was contained in a jar of ripe olives supplied by the hostess to her guests. The ultimate source and character of the poison remain for consideration.

THE TOXIC SUBSTANCE.

The poison in the olives must have been:

(1) Something inherent in the olives themselves;
(2) Something added during the canning process;
(3) Something added after the can was opened; or
(4) Something formed in the jar by the action of micro-organisms.

The first assumption need scarcely be considered in so staple a food as olives.

Concerning the second possibility, we know but little, since we are as yet ignorant of the exact procedure of canning. The olives, in question were packed by a firm bearing an excellent reputation and there seems to be no ground to doubt that reasonable care was observed in their preparation.

The jar in question was purchased on the evening of the banquet, and was taken directly to the club. It was delivered to a trusted waiter by a member of the party who gave instructions for serving. The waiter opened the jar at once, placed the olives in three dishes, washing those in two of the dishes, and placed the dishes on the table. There seems to have been little opportunity for anyone with malicious intent to poison the jar after its purchase, as has been suggested by some, and no reason to suspect that such a thing had been done. The possibility that the poison was a bacterial toxin will be considered in the discussion of the bacterial examination of the olives and brine.

Toxicity of the olives and brine.—Six olives and a small amount of brine from the original jar were recovered, a waiter having placed them in the ice box, where they remained until secured by a local investigator.

The 6 olives and brine were delivered to Dr. John G. Spenzer of Cleveland, a chemist, for examination. From Dr. Spenzer the State department of health secured 2 olives and about 5 cc. of brine.

The 2 olives when secured on September 3 were light brown in color, soft, considerably macerated, and had a putrid odor suggestive of feces. Chemical examination by Dr. Spenzer gave the following results:

Volatile poison, 0.
Irritant poison, 0.
Corrosive poison, 0.
Alkaloidal poison, 0.
Glucosidal poison, 0.
Putrefactive poison, 0.

A portion of turkey also submitted to Dr. Spenzer for examination gave entirely negative chemical and bacteriological findings.

Animal experiments.—(*a*) Inoculation Experiments: The authors used guinea pigs weighing from 250 to 300 grams throughout their animal experiments. An emulsion of one-half an olive in 10 cc. of sterile saline, given subcutaneously, proved lethal to guinea pigs in 1 cc. dose, while 0.5 cc. gave symptoms but recovery (Table IV).

TABLE IV.—*Toxicity of recovered olives.*

Guinea pig.	Received.	Amount, c. c.	Route.	Result.	Time later.
No. 1	½ olive in 1.0 c. c. saline	1	Subcutaneous	Death	24 hours.
No. 2	do	0.5	do	Ill but recovered	
No. 3	½ olive in 10 c. c. saline (control)	1	do	Not ill	50 days.

Varying amounts of brine were next injected subcutaneously into guinea pigs in doses varying from 1 c. c. to 0.001 c. c. These pigs all died in from less than 18 hours to 4 days. (Table V.)

TABLE V.—*Toxicity of recovered brine.*

Guinea pig.	Received.	Amount, c. c.	Route.	Result.	Time later.
No. 4	Olive brine	1	Subcutaneous	Death	18 hours.
No. 5	do	0.5	do	do	Do.
No. 6	do	0.1	do	do	31 hours.
No. 7	do	0.01	do	do	32 hours.
No. 8	do	0.001	do	do	96 hours.
No. 9	Olive brine (control)	1	do	Not ill	52 days.

A jar of ripe olives of the same brand and shipment as those used at the banquet furnished the material for controlling these experiments. The control pigs remained well.

(*b*) Feeding experiments: Two pigs, each forced to swallow 0.15 c. c. of brine left from the banquet, died on the third day following. A third pig, forced to swallow an uncertain amount of one of the two recovered olives, died also on the third day following. (Table VI.) The controls remained normal.

TABLE VI.—*Feeding experiments, recovered olives, and brine.*

Guinea pig.	Received.	Amount (c. c.).	Route.	Result.	Time later.
No. 10	Olive brine	0.15	Mouth	Death	70 hours.
No. 11	do	0.15	do	do	Do.
No. 12	Olive	0.5 ±	do	do	84 hours.
No. 13	Olive brine (control)	0.15	do	Not ill	48 days.
No. 14	Olive (control)	0.5 ±	do	do	Do.

(*c*) Sterile Filtrate: Three c. c. of the original brine, diluted to 20 c. c. with sterile saline, was filtered through a Berkefeld filter. The filtrate, which proved to be sterile to both aerobic and anaerobic cultures, was next injected subcutaneously into guinea pigs and proved to be highly poisonous. (Table VII.)

TABLE VII.—*Sterile filtrate, original brine.*

Guinea pig.	Received.	Amount (c. c.).	Route.	Result.	Time later.
No. 15	Olive brine filtrate	0.15	Subcutaneous	Death	25 hours.
No. 16	do	0.075	do	do	41 hours.
No. 17	Olive brine filtrate (heated)	0.15	do	Not ill	48 days.
No. 18	do	0.075	do	do	Do.

The recovered olives and brine had been mixed with tap water, exposed to air, dishes, fingers, etc., for several days, and were grossly contaminated with various organisms. It is apparent that the guinea pigs had not died of septicemia, however, since the sterile filtrate was also lethal. Moreover, autopsy was performed on each pig that died, and no evidence of septicemia was found in any case.

(*d*) Toxin Destroyed by Heat: The above-mentioned filtrate after heating to 80° C. for 30 minutes proved harmless. Similar doses in the "raw" occasioned death in 25 and 41 hours.

The pathological findings and clinical features will be referred to later.

Examination for anaerobic sporebearers.—Samples of the original olives and brine, heated to 60° C. for 60 minutes, following Dickson and Burke,[1] were inoculated, in varying dilutions, into deep tubes of molten beef infusion 1 per cent dextrose agar made 0.2 per cent alkaline to phenolphthalein. Following inoculation, the tubes were covered with liquid paraffin, cooled rapidly, and incubated at 37° C. and at room temperature. Within 48 hours colony formation was observed in the 37° C. tubes, followed by abundant gas formation and active fragmentation of the agar. At room temperature, growth could not be detected until the fifth day.

Suitable tubes were selected, broken across, and various colonies picked and transfers made to fresh tubes of beef infusion dextrose agar and into beef infusion dextrose broth for further study.

The broth transplants cultured anaerobically at 37° C. showed abundant growth at the end of 4 days. These tubes were tested for toxin by injecting 1 c. c. subcutaneously into guinea pigs. Several tubes showing a toxin lethal in this amount were selected for study.

Characteristics of the organism.—Morphologically the organism is a coarse bacillus varying from 2 to 6 microns in length, usually with rounded ends. It occurs singly, but occasionally in pairs. Motility, while present, is not vigorous. Under suitable conditions numerous terminal oval to round spores are found, which, being of greater diameter than the vegetative form, cause a terminal swelling. Young cultures are definitely, yet not strongly, Gram-positive. The organism stains well with the ordinary dyes, but takes the stain irregularly, barred forms often being found. The spores stain more faintly than the balance of the cell; however, spore-bearing organisms are often encountered where the whole cell stains faintly.

Culturally, the organism is a strict anaerobe. In our work, the organism was grown under oil or in the Novy jar in an atmosphere of hydrogen. Later, it was found by one of the writers (Story) that natural gas, such as is used in the laboratory, would answer for displacing the air, and permitted good growth. In the latter part of the work, gas was used for this purpose in place of hydrogen.

Growth is best at 37° C., but occurs at room temperature and at 20° C. after several days. Cultures have a characteristic odor suggestive of strong butter or cheese.

On meat infusion agar or meat infusion dextrose agar made slightly alkaline, colonies can be observed in from 3 to 5 days at 37° C.

[1] Jour. A. M. A., August, 1918, 518.

On dextrose agar, gas is formed and the agar is actively fragmented.

Gelatin is liquified at 20° C. in from 4 to 7 days with a diffuse growth.

Litmus milk is coagulated with decoloration of the litmus in from 2 to 3 days at 37° C. Later, partial peptonization occurs.

In beef infusion dextrose broth, vigorous growth with gas formation is seen at the end of 24 hours at 37° C., and later at room temperature.

Dextrose, saccharose, lactose, and mannite are fermented with gas and acid formation.

The different strains of *Bacillus botulinus*, as described by various authors, are found to vary with reference to their cultural reactions, which, it may be said, are imperfectly understood. This particular organism differs from several described strains in its action on milk and sugars.

From its morphology, toxin formation, and growth characteristics, together with the symptoms and pathological lesions produced, this organism is considered to be a strain of *Bacillus botulinus*. This opinion has been confirmed by Sisco, of the Harvard laboratories.[1]

Growth on olive media.—Ripe, unspoiled olives and brine of the same brand as that of the original jar were used for this purpose. The olives were chopped, tubed, covered with brine, and nothing else was added. The tubes were autoclaved at 15 pounds for 30 minutes, cooled rapidly, inoculated, coated with oil, and incubated at 37° C. and at room temperature.

After 3 days at 37° C. the brine was clouded and there was moderate gas formation, bubbles accumulating in the ground olives at the bottom of the tube. The organism produced abundant spores on this medium and gave the peculiar rancid odor of *Bacillus botulinus*. Tubes grown at room temperature and at 37° C. were found after 9 days to contain a powerful toxin. On chemical examination the olive liquor was found to be a weak brine, having 2.87 grams of solids per 100 cc., of which 1.67 grams were sodium chloride, evidently too little to inhibit *Bacillus botulinus*, as the organism was found to grow well on meat infusion dextrose broth containing salt to 3 per cent, and still grew, though less vigorously, in a medium with 6 per cent sodium chloride.

Effect of light.—The effect of light on this organism has not been fully studied; but it does not seem to be important in connection with this case, since in a jar of closely packed olives covered by a dark-colored brine there would seem to be but little opportunity for light to operate. The most important condition affecting the growth of

[1] Personal communication.

the organism seems to be the presence or absence of oxygen. A vacuum sealed jar may be expected to furnish the required anaerobic conditions. The formation of gas from within, or a defective seal, might account for the fact that the vacuum had been destroyed in this particular jar, for it will be recalled that the lid came off easily. Had the seal been defective, however, and allowed air to enter, it is still probable that *Bacillus botulinus* could have grown; for the presence of air would have encouraged the growth of the usual putrefactive organisms which are known to utilize the free oxygen from media and thus produce conditions favorable for the growth and toxin formation of *Bacillus botulinus*.[1]

Spore formation.—Spores were found at times in nearly all media on which the organism was observed to grow, but were especially numerous and constant in the olive medium.

Resistance to heat.—Tubes of meat infusion dextrose broth or agar, seeded with *Bacillus botulinus* from an olive culture possessing numerous spores, showed the latter to be quite resistant to heat. Tubes heated to 100° C. for 30 minutes in the Arnold sterilizer, when incubated at 37° C., showed growth and gas formation on the fourth day. Tubes heated for longer periods at 100° C., or autoclaved at 15 pounds for 15 minutes, have shown no growth after 14 days.

Toxin formation.—Tubes of meat infusion dextrose broth and of the above-mentioned olive medium, when seeded with the mixture of organisms from the original toxic olives, produced a strong toxin in 8 days. In pure culture a strong toxin was also formed in olive and other media.

In order that a standard toxin might be obtained, flasks of beef infusion 1 per cent dextrose broth, slightly alkaline, were inoculated with pure culture of *Bacillus botulinus*, covered with oil and incubated at various temperatures. Tubes grew best at 37° C. and with more rapid toxin formation, a 9-day-old culture developing a toxin approximately 200 times as strong as an 11-day-old culture grown at room temperature. The sterile filtrate from this 9-day-old 37° C. culture proved lethal to guinea pigs in 0.00,005 cc. doses when administered intraperitoneally. This toxin kept in the icebox was used throughout the following experiments.

The effect of alcohol on the toxin.—That alcohol might possess the property of destroying *Bacillus botulinus* toxin was suggested by the epidemiological data. Two cases, it will be remembered, who recovered after eating one and two olives, respectively, had partaken more or less freely of alcoholic drinks during the evening.

In testing for the effect of alcohol on the toxin, various doses of toxin diluted to 1 cc. with sterile saline, were mixed with 0.5 cc. of

[1] Von Ermengem, Shippen, and others.

95 per cent alcohol, thus giving in the test tube a mixture of approximately 32 per cent alcohol. The mixtures were allowed to remain for several minutes in the tube, with frequent shaking to prevent any precipitate which might form from settling. The mixtures were then injected either subcutaneously or intraperitoneally into guinea pigs. It was found possible in this manner to protect guinea pigs against 20 times the lethal dose of raw toxin. (See Table VIII.) The effect of alcohol on toxin given by mouth and its possibilities as a therapeutic agent are being studied and will be reported on later.

TABLE VIII.—*Results of administration of alcohol-toxin mixtures.*

Guinea pig.	Received—				Route.	Result.	Time later.
	Toxin.		Alcohol.				
	Amount.	Number of fatal doses equivalent to—	Amount.	Per cent in mixture.			
	cc.		cc.				
No. 26	0.01	200	0.5	3.33	Intraperitoneal	Death	18 hours.
No. 27	.01	200	.5	8.33	do	do	Do.
No. 28	.01	200	.5	8.33	Subcutaneous	do	Do.
No. 29	.01	200	.5	32.0	do	do	20 hours.
No. 30	.002	40	.5	32.0	do	do	49 hours.
No. 31	.001	20	.5	32.0	do	Recovered	20 days.
No. 32	.001	20	.5	32.0	Intraperitoneal	Death	[1] 4 days.
No. 33	.001	20	.5	32.0	Subcutaneous	Recovered	18 days.
No. 34	.0005	10	.5	32.0	do	do	Do.
No. 35	.0002	4	.5	32.0	do	do	Do.
No. 36	.0002	4	.0(control)		do	Death	4 days.

[1] Pneumonia.

SEROLOGICAL EVIDENCE.

Forty-five days after the fatal meal, serum was collected from three recovering patients. Agglutination tests by both microscopic and macroscopic methods showed the serum from the recovering patients to be agglutinative for the isolated organism in dilutions of 1:100; this, however, was no higher than was secured in controls with normal serum. (Table IX).

TABLE IX.—*Agglutination.*

Serum of patients—	Dilutions.					
	1:20	1:40	1:80	1:100	1:150	1:200
C. B. ♀	+	+	+	±	−	−
C. O. ♂	+	+	+	+	−	−
Control	+	+	+	+	−	−
Do	+	+	+	±	−	−

Antitoxin.—Varying amounts of toxin were mixed with 1 cc. of serum from the recovering patients, and the mixture left to stand in the test tube for several minutes before injection. The mixtures were given subcutaneously and proved as lethal as the corresponding amounts of toxin mixed with normal serum (Table X).

TABLE X.—*Effect of toxin-serum mixtures.*

Serum from patient—	Guinea pig.	Received—			Route.	Result.	Time later.
		Toxin.		Serum.			
		Amount.	Lethal dose.				
		c.c.		c.c.			
C. B. ♀	No. 40	.0001	2	1	Subcutaneous.	Death	(?).
C. B. ♀	No. 41	.0005	10	1	do	do	Second day.
C. B. ♀	No. 46	0	0	1.5	do	No effect	16 days.
C. O. ♂	No. 42	.0001	2	1	do	Death	Second day.
C. O. ♂	No. 43	.0005	10	1	do	do	24 hours.
C. O. ♂	No. 47	0	0	1	do	No effect	16 days.
I. W. G. ♂	No. 44	.0001	2	1	do	Death	Second day.
I. W. G. ♂	No. 45	.0005	10	1	do	do	25.5 hours.
X (control)	No. 48	.0001	2	1	do	do	Second day.
Y (control)	No. 49	.0001	2	1	do	do	Third day.

While agglutination and antitoxin formation against various strains of *Bacillus botulinus* have been demonstrated in experimental animals —goats, horses, and mules—by various workers, their production has been attended with considerable difficulty. We have been unable to find a case of botulism in man where serological tests were successful in identifying the organism. The patients from whom blood was received were I. W. G., a mild case, whose only symptoms were weakness, some change in his voice, and a slight difficulty of speech. He was well at the time blood was secured. The other two patients, Mrs. C. B. and C. O., were quite severe cases, and while the eye, throat, and paralytic symptoms had practically disappeared, there was still a profound weakness in each case. An attempt to demonstrate the presence of free toxin in the circulating blood of these patients was made by injecting 1.5 cc. of serum into the peritoneal cavity of guinea pigs. No ill effects developed from this dose. Larger amounts were not used as the serum was not available. Complement fixation tests were not made.

Growth and toxin formation in animals.—Working with his original cultures, von Ermengem failed to produce toxin at 30° C. and above, and concluded from this fact that *Bacillus botulinus* was unable to develop its toxin in a warm-blooded animal. Several strains, including the one under investigation, have been found by various workers to have their optimum growth and toxin formation at 37° C.

Following some suggestive work by Thom and others,[1] a guinea pig was given, subcutaneously, some 300,000,000 *Bacilli botulini* from a flask containing powerful toxin and numerous spores. The organisms before injection were freed of toxin by heating to 80° C. for 30 minutes. The animals were still well 26 days after the injection.

Heated cultures, force fed and given on grass and feed, likewise failed to cause any symptoms in guinea pigs; cultures, however, showed the presence of viable organisms following the heating. (Table XI.)

TABLE XI.—*Effect of spores on guinea pigs, injected subcutaneously and fed.*

Guinea pig.	Millions of organisms received.				Route.	Result.	Time later.
	Heated to 80° 30 minutes.	Washed 12 times.	Washed 14 times.	Washed on filter.			
No. 50	300				Force fed	Not ill	26 days.
No. 51	300				Subcutaneous	do	Do.
No. 54		300			do	Died	18 hours.
No. 55		(?)			Fed on grass	Not ill	25 days.
No. 57			300		Subcutaneous	Died	23 days.
No. 58			(?)		Fed on grass	Not ill	Do.
No. 61				1200	Force fed	Died	Third day.
No. 62				120	Subcutaneous	do	Fourthday
No. 63				12	do	Not ill	20 days.
No. 64				1.2	do	do	Do.
No. 65				(?)	Fed on grass	do	Do.

Organisms from a culture possessing powerful toxin were next washed in distilled water by agitating, centrifugalizing, decanting, and repeating for 12 separate washings in order to free of toxin. A pig injected subcutaneously with approximately 300,000,000 washed organisms was found dead in its cage some 18 hours later. A second culture similarly washed for 14 times but with greater agitation each time, likewise proved lethal when administered subcutaneously. When fed to animals on grass, however, there was no ill effect.

A culture was next washed on a Berkefeld filter by passing 800 cc. of sterile saline through the filter. The organisms were recovered by reversing the current. One pig which received 120,000,000 organisms injected beneath the skin died in 4 days, while two others which received 12,000,000 and 1,200,000, respectively, remained well.

A guinea pig force fed with 1,200,000,000 washed organisms died in 70 hours, while another given the organisms on grass and meal failed to show any symptoms.

It is seen that the organisms are difficult to free from toxin by washing. However, they can be freed to the extent that large numbers may be injected subcutaneously or fed to guinea pigs with no symptoms following.

[1] Jour. A. M. A., Sept. 20 1919.

The epidemiological data, moreover, would seem to indicate that the organism had not grown and produced toxin in the human cases. For had the bacilli swallowed with the olives been capable of growing and producing toxin in the alimentary tract, it seems that some of the people who ate small amounts and were but little affected would have developed serious symptoms. There is, however, on the other hand, a remarkable correspondence between the amounts eaten and the severity of the illness. A possible explanation of this fact might be sought in assuming that antitoxin was produced by the individual more rapidly than the organisms formed toxin. It will be remembered, however, that no antitoxin could be demonstrated in the blood of recovering patients. An effort was made to determine the number of *Bacilli botulini* found in one of the recovered olives. A carefully weighed portion was emulsified in saline heated to 60° C. for 60 minutes, and varying amounts were "plated" into deep tubes of meat infusion agar, incubated, and colonies determined. It was thus calculated that this olive contained as a minimum 1,300,000 bacilli, presumably spore bearers, while in the raw there were possibly several times this number of nonspore-bearing *Bacilli botulini*. It would seem that a bite of olive containing this number of viable organisms, if capable of multiplying and forming toxin in the alimentary tract, should have caused serious infection. The number of cases, however, are too few to permit conclusions, and it is not possible to say that the organisms might not produce toxin in a tonsillar crypt, a decayed tooth, the intestinal tract, or other locations where anaerobic conditions might at times prevail.

TABLE XII.—*Signs and symptoms.*

Patient.	Date of first symptoms.	Hours from dinner to death.	Signs and symptoms.																																	Remarks.
			Headache.	Thirst.	Double vision.	Dim vision.	Weakness.	Difficult speech.	Aphonia.	Pain.	Colic.	Dizziness.	Vomiting.	Diarrhœa.	Constipation.	Anorexia.	Congestion in throat.	Pulse rate.	Blood pressure.	Respiration.	Ptosis right eye.	Ptosis left eye.	Inability to focus.	Dysphagia.	Paresthesia.	Hyperesthesia.	Dilitation of pupils.	Pupilary reflex.	Knee jerks.	Babinsky.	Sensorium.	Anuria.	Retention or incontinence.	Highest temperature.	Manner of death.	
R. J. ♂ ...	8-24	54	0	0	+	0	+	+	0	(1)	0	0	+	0	(2)	+	0	95	?	22	0	0	?	+	?	?	+	N.	N.	0	N.	?	?	98.6	Resp.	Vomited 26 hours after meal.
I. W. G. ♀	8-24	55.5	0	0	+	+	+	+	+	0	0	+	0	0	0	0	sl.	84 96	?	?	+	+	+	+	0	0	0	N.	N.	0	N.	0	0	98	Resp.	
C. C. W. ♂	8-23	59.5	0	0	+	+	+	+	+	0	0	+	+	0	0	+	sl.	78 96	120 80	20	0	0	+	+	+	0	0	slow.	N.	0	N.	0	0	98	Resp.	Vomited 4 hours after meal.
J. C. S. ♂ ..	8-24	69	0	+	+	+	+	+	0	sl.	0	+	+	0	+	0	sl.	60 120	120 ?	18	+	+	+	+	0	0	+	slow.	N.	0	N.	+	0	98.6	Resp.	Slight abdominal pain. Much thick mucus in throat.
F. Mc. A ♂	8-25	75	0	+	+	+	+	+	+	(1)	sl.	+	+	0	+	+	sl.	70 80	?	18 20	+	+	+	+	0	0	+	N.	N.	0	N.	0	0	N.	Resp.	
J. C. S. ♀ ..	8-25	86.5	sl.	+	+	+	+	+	0	sl.	0	+	+	0	(2)	0	0	5 140	126 ?	?	+	+	+	+	0	0	+	abs.	N.	0	N.	0	0	99	Resp.	Vomited after a dose of castor oil.
W. F. S. ♀	8-25	174	0	0	+	sl.	+	+	+	0	0	sl.	0	0	0	0	sl.	90 158	115 ?	18 30	0	sl.	+	+	0	0	+	slow.	N.	0	N,	0	0	99	{cardiac {+ resp.	
C. O. ♂ ...	8-26	.....	0	+	+	+	+	+	+	(1)	0	+	+	0	+	0	sl.	60 80	120 ?	18 24	+	+	+	+	0	0	+	+	N.	0	N.	0	0	98.4	. .... .	Vomited, 60 hours after meal.
L.H.B. ♀ .	8-24	.....	+	0	0	+	0	+	0	0	0	+	0	0	0	0	0	92	?	N.	+	+	+	+	0	0	0	N.	N.	0	N.	0	0	99	.....	Infection of conjunctivæ.
C. B. ♀ ...	8-24	.....	+	+	+	+	+	+	0	0	0	+	+	0	+	+	sl.	112	?	N.	+	+	+	+	0	0	+	slow.	N.	0	N.	0	0	98.6	.....	Vomited third day.
L.H.B. ♂ .	8-24	.....	+	0	+	+	0	+	0	0	0	+	0	0	0	0	0	82	?	N.	0	0	+	+	0	sl.	0	N.	N.	0	N.	0	0	98.6	.....	
I. W. G...	8-27	. ....	0	0	0	0	+	+	0	0	0	0	0	0	s.	+	0	N.	?	N.	0	0	0	0	0	0	0	N.	N.	0	N.	0	0	98.6	... .	
C.C.W. ♀.	?	.....	+	0	+	0	+	+	0	0	0	0	0	0	+	0	0	90 100	?	N.	0	0	0	0	+	+	0	N.	N.	0	N.	0	0	N.	....	Sensitive to light.
C. B. ♂ ...	8-26	.....	0	+	0	+	+	?	0	0	0	0	+	0	+	+	0	N	?	N.		0	+	0	0	+	0	N.	N.	0	N.	0	0	N.	.....	Vomited six times during first week.

[1] Throat. [2] Purgatives given. N.=Normal. Sl.=Slight.

SYMPTOMATOLOGY.

The symptoms in the 14 cases were very similar though varying in some respects mainly in severity. A summary of physical signs and symptoms is given in Table XII.

The case of Mrs. W. F. S., as reported by Dr. L. F. Mutschmann, is given in detail as follows:

I first saw the patient on August 25, 1919, 52 hours after the dinner. She complained at that time of slight headache, diplopia, moderate degree of dimness of vision, and a very slight vertigo.

History.—Patient stated that she had attended the dinner at Canton on August 23, and that she had been in good health prior to this time. She recalled distinctly that on biting into a ripe olive it tasted spoiled. She swallowed this portion of olive and laid the rest aside, as the taste was not agreeable.

Examination.—On examination I found her vision to be somewhat impaired, pupilary reflexes sluggish, pupils fairly dilated, and a partial inability to rotate the left eye externally; also a slight ptosis of the left eyelid. Her temperature was normal; pulse 85; respiration 18. The blood pressure was 110, systolic; 70, diastolic. Mucous membranes of the nose and throat were only moderately congested, as were the conjunctivæ. There was at this time no audible change in speech as far as I was able to detect, nor in deglutition. The lungs were negative, the heart gave a slightly accentuated second sound. The abdomen was normal in contour, there being no distention or rigidity. The bowels and kidneys were acting normally. Patellar reflexes were normal; Babinsky absent.

August 26, 1919: The following morning, August 26, there was some embarassment of deglutition, and, to a less extent, in articulation. The pulse was about the same as on the previous day. Temperature, 98; pulse, 90; respiration, 20. The patient was able to take nourishment and felt fairly comfortable, with the exception of a slight vertigo and headache when she kept her eyes open for any length of time. This difficulty in deglutition and speech was more marked on the night of August 26.

August 27, 1919: On the morning of August 27, patient was able to rinse out the mouth, but unable to swallow; had fairly good control of the tongue during speech. There was no acute dryness of the mouth, but she complained of a slight pain and rather distressing, burning sensation in the abdomen. During the afternoon she complained of some colicky pains in the region of the lower abdomen, which disappeared after expelling a goodly quantity of brown fluid stool. Her temperature at 4 o'clock that afternoon was 97; pulse, 85; respiration, 24. By 9 o'clock that evening the patient was unable to gargle, and began to complain of pain and a feeling of constriction in the throat, which gradually increased and distressed her greatly.

August 28, 1919: The morning of August 28 found the patient in practically the same condition, but rather drowsy and complaining of dryness and a sensation of mucous clinging in her throat, which she was unable to swallow or deliver through the mouth. She was at this time unable to protrude the tongue beyond the lips. At 7

p. m. she was relieved quite suddenly of the dryness in the throat and mouth and was able to move the tongue more freely, and wanted to try to take fluids but was unable to swallow them. At 10 p. m. she complained of a pain in the region of her heart, which traveled through the left axilla into the back and lasted about five minutes. During this time she experienced slight difficulty in breathing and became very restless.

August 29, 1919: At 6 o'clock on the morning of August 29 her chief complaint was that her throat felt very dry and raw, and that she felt extremely weak and had a sensation of her throat closing up. Change of position to her right side seemed to give her some slight relief. At noon of the same day her face became flushed, and after an hour of sleep she awoke with an increase in the choking sensation which was accompanied by slight cyanosis of the face. She was very restless. These choking sensations occurred after each short interval of sleep during the remainder of the day. During the following night she was very much fatigued and slept about an hour in all. I had received some *botulinus* serum from the agricultural department of the University of Illinois, and had given her a desensitizing dose at 9 o'clock on August 29. There being no apparent reaction, she was given 5 cc. hypodermically. Again at 4 o'clock she was given 5 cc. After each injection she perspired profusely and complained of feeling hot and very weak, but within an hour seemed to recover and felt improved.

August 30, 1919: On the morning of August 30 her temperature was 98; pulse, 90; respiration, 22. Her systolic blood pressure was 100; diastolic 70. She was given another 5 cc. of the serum. At evening she was resting rather quietly but constantly trying to clear her throat. Her temperature at noon was 98; pulse, 90; respiration, 24. By 2 o'clock her pulse was 118, her body felt cold and was covered with a clammy perspiration. Toward evening her respirations increased to 28 and were shallow and slightly irregular. The pulse was 126 and she was quite cyanotic, but appeared to be resting, though very weak. The respiration gradually became more shallow and the pulse more irregular.

August 31, 1919: On the morning of August 31, her pulse was 158; respiration, 24. She was too weak to move in bed and unable to talk; the cyanosis was gradually spreading; her body was bathed in a profuse, cold perspiration. Respiration ceased at 2.15, cardiac failure occurring first.

On my first visit I prescribed large doses of magnesium oxide and hypodermics of strychnia—grains 1/40 every three hours; hypodermics of camphorated oil were added to this toward the latter part of the illness. After she was unable to take fluids by mouth she was given 500 cc. of saline by the "Murphy method" every three hours, which she retained on the whole very nicely.

She was not troubled with constipation nor diarrhea at any time during the illness. Nutritive enemas were given and occasionally black coffee and small quantities of brandy.

Symptomatology in animals.—In guinea pigs the symptoms appear in from 6 to 48 hours, or even longer, according to the dosage, following subcutaneous injection. The symptoms are slower in onset where the toxin is fed.

With the onset of illness the animal sits as though cold, the hair is roughened, and the flanks are sunken. Respiration is soon disturbed; it becomes slower than normal and is attended with considerable effort. This continues until there is complete diaphragmatic paralysis. There is great weakness, and the animal lies on its abdomen with extremities extended. The cornea appears dry, and often the animal is unable to wink. The neck is usually completely paralyzed. No dribbling of saliva has been observed in guinea pigs. In other cases the paralysis and weakness seem confined to the posterior part of the animal; the head is held up and the animal is able to wink normally. Temperature is usually subnormal.

Guinea pigs in the last stage of poisoning, etherized and the abdomen opened, showed the diaphragm to behave as a flaccid membrane. The stomach is usually found dilated, and peristalsis of the organ is not observed even after pinching or pricking. The small intestine is found empty, or nearly so, and in active peristalsis. The large intestine is usually found packed with solid contents and devoid of peristalsis. The heart continues to beat after respiration has ceased.

Cats seem relatively more resistant to the toxin than guinea pigs. A cat given 0.5 cc. of powerful toxin showed no symptoms until the third day, when three dead kittens were aborted. On the fourth day there was noted a dribbling of saliva and weakness of hind parts. This progressed until there was inability to stand or raise the head. The pupils reacted to light, and winking was normal. Respiration was easy but shallow. There was inability to mew. There was no fever, and constipation was marked. The cat was anesthetized on the sixth day, and findings were similar to those in the guinea pigs.

PATHOLOGY.

Two coroner's autopsies were performed prior to this investigation, one complete and the other confined to the abdomen. The ligated stomach, a portion of the intestine, a kidney, and piece of liver from the case of R. J., together with the same organs and a piece of brain from F. McA., were submitted to Dr. John G. Spenzer, of Cleveland, for chemical examination. The various organs are said to have been quite normal in appearance. No material suitable for microscopic study is available. Dr. Spenzer found "no mechanical, volatile, irritant, corrosive, metallic, alkaloidal, glucosidal, or putrefactive poison, even in traces," in the organs examined.

Animal pathology.—The organs and peritoneum of guinea pigs appeared quite normal to inspection, with the exception of a generalized congestion which was present without exception in the animals examined. The veins and arteries stand out prominently, and the stomach and large intestine are usually distended.

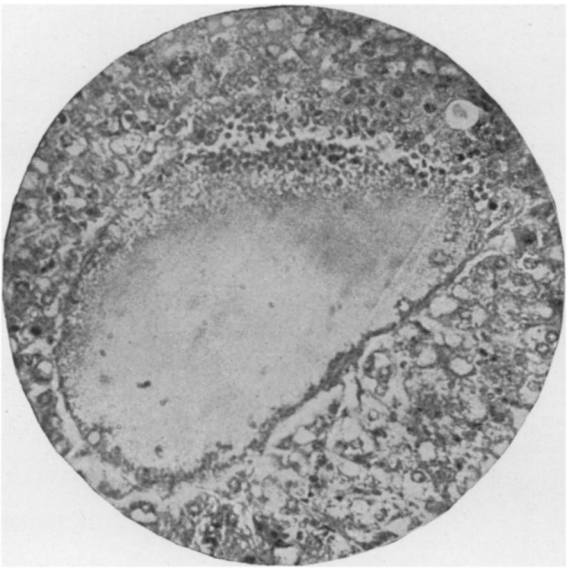

Fig. 2.—Hyaline thrombus occurring in vein of the liver, showing red blood cells and leucocytes at margin. From case No. 39, which received 0.001 cc. of a Berkfeld filtrate of a 9-day broth culture, together with 1 cc. of the serum from a patient who had recently recovered from botulism. Death of the guinea pig occurred in 24 hours.

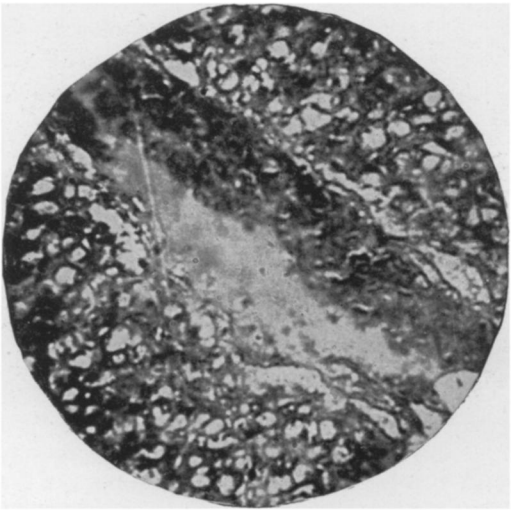

Fig. 3.—Partial hyaline thrombus occurring in a vein of the liver, showing admixture of red blood cells. From case No. 24, which received 0.0001 cc. of 9-day broth culture. Death in 24 hours.

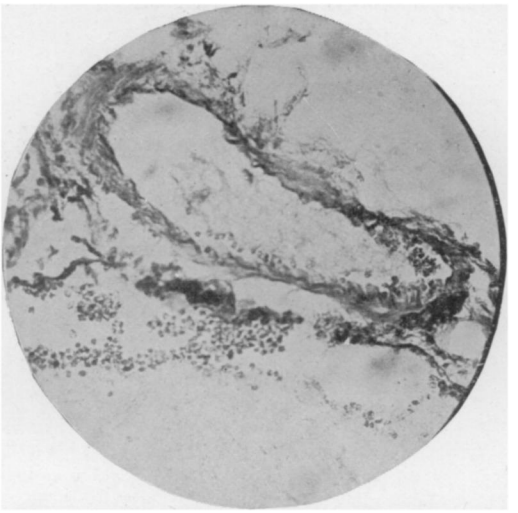

Fig. 4.—Fibrinous thrombus occurring in an artery and vein of the kidney in case No. 25, which received 0.0001 cc. of a Berkfeld filtrate of a 9-day broth culture. Death in 17.5 hours.

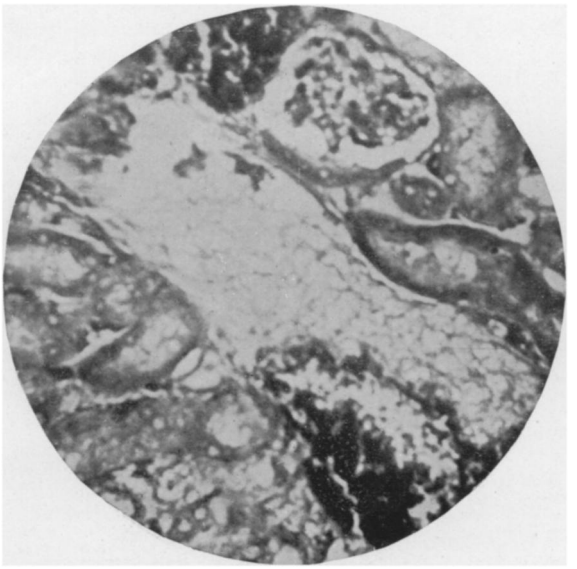

Fig. 5.—A higher magnification of a fibrinous thrombus occurring in an intertubular vein of the kidney of the case shown in Fig. 4.

The pleura and thoracic organs show the same generalized congestion. Pneumonia was found in two cases.

The brain appears normal except that the meningeal vessels are distended.

Macroscopic hemorrhages were present in the lungs of one animal examined in this series.

Microscopic animal pathology.—The inoculations and autopsy examinations were made in the laboratory of the Ohio State department of health. The tissues, after being placed in Zenker's fluid or formalin, were sent to the laboratory of pathology of the Ohio State University, where they were examined by Dr. Ernest Scott, head of the department of pathology, whose report follows:

The tissues of this series consist of the visceral organs and the brains of 18 guinea pigs and 1 cat. The most striking feature of the microscopical picture is the intense hyperemia present in all of the specimens examined. This congestion involves all of the vessels, being possibly a little more marked in the veins, but present always in the arteries and in the capillaries as well. Without exception the ventricles of the heart are filled with blood. Associated with this congested condition of the organs there is also a uniform and almost equally conspicuous degeneration of the functional cells of the liver, kidneys, adrenal glands, and heart muscle. This parenchymatous change is so marked in some instances that no normal cells can be found. In a few instances the degeneration has progressed until karyolysis and cytoplasmic disintegration are well marked. In the heart muscle swelling of the fibers with loss of striation and hydrops are frequently seen.

Dickson, in his monograph on "Botulism," [1] notes that thrombosis of the vessels is of very constant occurrence in animals suffering from botulinus poisoning. So constant, in fact, is this thrombosis that the author states that "Thrombi are so uniformly present and are so characteristic in appearance that they may be considered pathognomonic of botulism."

In discussing these thrombi, he divided them into two rather distinct types: In the first type the thrombus consists of "dense masses of fibrin arranged in thick bands and have many polymorphonuclear leucocytes enmeshed between these strands"; the second variety, or that which the author calls the "prethrombus stage," consists of "hyaline masses or loose bunches of fibrin, in which leucocytes and red blood corpuscles may be enmeshed."

The thrombi encountered in the series under discussion have been altogether of the second class or "prethrombus" type, the thrombi being chiefly of the solid or hyaline variety, with the occurrence of a definite fibrinous network within the vessels in only a small percentage of the cases. Of the 18 guinea pigs examined in this series

[1] Rockefeller Monograph No. 8.

(Table XIII), 14 showed the presence of such thrombi. In some, these thrombi were very definite and easily seen; in others, careful search was necessary to reveal them. Such thrombi were found most commonly in the liver, 14 of the cases showing involvement of this organ. There were 3 cases in which the vessels of the brain or meninges were involved, 3 cases in which the vessels of the kidneys were thrombosed, and 3 in which the vessels of the lung were involved. Sections made from the tissues of the cat showed that thrombi were not only more numerous, but that they more nearly resembled the thrombi of the first class described by Dickson, being larger, more definitely formed, and showing numerous leucocytes and red blood cells entangled in their substance. There are also present in this case many thrombi of the simpler, more purely hyaline type.

The fact that in Dickson's series of 30 guinea pigs, thrombus formation occurred in only 1 case in the first 24 hours, and that "prethrombi" occurred in 6 cases within this time may explain the presence of such a large percentage of the "prethrombus" type in the present series. It will be observed from Table XIII that of the guinea pigs used only 5 lived longer than 30 hours, while in the case of the cat, where more definite thrombosis is seen, the time elapsing before death was 6 days.

The rapid and uniformly fatal termination in these cases would indicate that the toxin produced by this strain of bacillus was of high virulency. This is further indicated by the fact that 0.00005 cc. of a Berkfeld filtrate killed the animal inoculated in 3 days.

The occurrence of hemorrhage was neither a constant nor a conspicuous factor in any of the series examined, occurring in only 3 of the cases; twice in the meninges at the base of the brain and once in the subpleural tissues of the lung.

The study of this brief series of animals tends to confirm Dickson's observation that the occurrence of thrombosis within the vessels is of great value in the diagnosis of this condition.

It will be noted (Table XIII) that the animals which died after being fed or injected with the original recovered olives and brine showed the characteristic lesions of botulism, similar to those produced by the organism isolated from the original toxic materials.

Table XIII shows the results of this study. In the investigation of cases of food poisoning where animal inoculations are made, the presence of thrombosis accompanied by hyperemia and parenchymatous degeneration should immediately suggest the presence of *Bacillus botulinus*. Special staining methods for the detection of the finer nuclear and granular changes of the brain cells were not applied.

TABLE XIII.—*Results of experiments on 18 guinea pigs.*

No.	Dose.	Time before death.	Hyperemia.	Thrombosis.	Parenchymatous degeneration.	Hemorrhage.
20	0.5 cc. 11-day broth culture (room temperature).	2 days	+	+	+	+
39	0.001 cc. Berkfeld filtrate (1 cc. serum from patient C. O.).	24 hours	[1] +	+	+	0
21	0.00005 cc. Berkfeld filtrate (9-day 37° C. broth).	3 days	+	+	+	+
24	0.0001 cc. broth culture (9-day 37° C.)	24 hours	+	+	+	0
25	0.0001 cc. Berkfeld filtrate (9-day 37° C. broth).	17.5 hours	+	+	+	0
37	0.001 cc. broth culture (9-day 37° C. culture).	16 hours (found dead).	+	+	+	0
38	0.005 cc. broth culture (9-day 37° C. culture).	do	+	+	+	0
22	0.01 cc. broth culture (9-day 37° C. culture).	do	+	+	+	0
19	2 cc. Berkfeld filtrate (8-day room temperature).	3 days	+	+	+	0
23	1 cc. broth culture (4-day 37° C. culture).	24 hours	+	+	+	0
23a	1 cc. broth culture	do	+	+	+	0
5	0.5 cc. original olive brine (fed)	18 hours	+	0	+	0
68	0.6 cc. olive media brine (9-day 37° C. culture).	24 hours	+	0	+	0
16	0.025 cc. Berkfeld filtrate (original olive brine).	41 hours	+	0	+	0
7	0.01 cc. brine from original olives	32 hours	+	0	+	0
6	0.1 cc. brine from original olives	31 hours	+	+	+	0
69	0.5 cc. olive media brine (9-day 37° C. culture).	18 hours	+	+	+	0
1	Suspension of original olives	24 hours	+	+	+	+

[1] Not marked in brain.

DIAGNOSIS.

That a single case of botulism may offer difficulty in diagnosis is quite apparent. In the present outbreak, as is usual, the individual cases were most puzzling until the occurrence of poisoning in others of the same group made the matter clear. Individual cases were early mistaken for mushroom poisoning, wood alcohol poisoning, ethyl alcohol poisoning, cerebral hemorrhage, cerebral lues, and hysteria. Other conditions which arise for differentiation are asthenic bulbar paralysis, toxic amblyopias, rabies, diphtheria, plant alkaloid poisoning, ptomaine, poliomyelitis, cerebrospinal meningitis, trembles, and encephalitis lethargica.

PROGNOSIS.

The mortality in different outbreaks has varied and has been as high as 100 per cent; but it is most often in the neighborhood of 50 per cent. In cases which escape death, recovery is usually complete, but it may require weeks or even months in the more serious cases. Broncho-pneumonia is the complication most feared. Weakness was the symptom slowest in disappearing in the cases of nonfatal poisoning herein considered.

TREATMENT.

The mortality from botulism is practically as high to-day as formerly, which indicates the unsatisfactory status of our knowledge of treatment. Dickson, quoting Muller, advises emesis of lavage even

after several days, as it is not unusual to find portions of the poisonous food retained in the stomach at the end of this time. Active purgation should be obtained and the colon irrigated. Patients should be kept in bed and as free from excitement as possible. Simple nourishing food and water should be given, but the danger of aspiration pneumonia must be remembered. Water is best given by rectum or subcutaneously when there is difficulty in swallowing.

Strychnia is recommended as valuable in improving the action of the damaged nervous system. Cardiac and other stimulants should be used as indicated. Antitoxin, if available, it is hoped might prove useful, but it probably must be given early to be effective. There are no available records of its successful use except in animals.

The limited evidence of the present outbreak would seem to indicate that alcohol, when given early, may be of value in lessening the symptoms, probably by destroying the toxin.

PREVENTION.

1. The ideal of prevention would be a process of canning which effectually kills all spore-bearing organisms. However, the great resistance of certain strains of *Bacillus botulinus* to heat and other agencies, as shown by Burke [1] emphasizes the danger that a few spores may occasionally survive almost any process of canning.

2. Thorough cooking of all canned goods before serving or sampling would render foods infected with *Bacillus botulinus* harmless, in so far as the presence of preformed toxin is concerned.

3. The rejection of canned foods which show even minor changes of taste, odor, or consistency. Several of the above patients ate of the olives even though they tasted "off."

SUMMARY.

1. The epidemiological investigation points to the ripe olives as the vehicle of the poison.

2. The olives and brine were found to be highly toxic for animals, both when fed and when injected.

3. The organism isolated from the olives and brine seems, from its morphology, cultural characteristics, toxin formation, and from the symptoms and pathological lesions produced, to be a strain of *Bacillus botulinus*.

4. Antitoxin and agglutinins could not be demonstrated in the blood of recovering patients 45 days after the dinner.

5. Alcohol has the property of neutralizing the toxin when mixed *in vitro*.

6. It would seem that *Bacillus botulinus* does not produce its toxin under usual conditions in a warm-blooded animal.

[1] Jour. A. M. A. Jan., 11, 1919.

SUPPLEMENTARY NOTE.—The authors later succeeded in securing some *Bacillus botulinus* antitoxin from Dr. John Buckley, Chief of the Pathological Division, Bureau of Animal Industry, U. S. Department of Agriculture. This antitoxin was prepared against the Boise strain of *Bacillus botulinus* and was found to be protective for guinea pigs injected with toxin formed by the organism isolated from the olives.

Pig No. 80, given intraperitoneally ½ cc. undiluted toxin, followed by ½ cc. antitoxin.

Pig No. 81, given intraperitoneally ½ cc. undiluted toxin, followed by 1/20 cc. antitoxin.

Pig No. 82, given intraperitoneally ½ cc. undiluted toxin, followed by 1/200 cc. antitoxin.

Pig No. 83 (control), given intraperitoneally ½ cc. undiluted toxin; no antitoxin.

Pig No. 83 (control) found dead in less than 12 hours.

Pig No. 82 showed typical symptoms on second day and was found dead on third day.

Pigs No. 81 and No. 80 have shown no ill effects and are well at end of fifth day.

One half cc. of toxin represented 200 lethal doses for guinea pigs when tested one month previous to this experiment. The toxin had been kept in the ice box during this interval.

PRECAUTIONARY MEASURES TO PREVENT LEAD POISONING.

The Office of Industrial Hygiene and Medicine of the United States Public Health Service has recently concluded a survey of the pottery industry, located chiefly in Trenton, N. J., and East Liverpool, Ohio. The survey was made with particular view to determining the extent of lead poisoning in this industry, and to give oral and written advice and precautionary instructions.

Approximately 2,000 men were given physical examinations during this survey. Where any pottery worker was found to be suffering from lead poisoning, even to the slightest degree, he was informed as to his condition and was given treatment and advice. Where any prominent physical defect was discovered, the worker was informed relative to the defect, and consultation with a physician was advised.

As a result of the physical examinations conducted by the medical officers of the Service, a number of cases of lead poisoning were discovered, and it was considered advisable to call the attention of all pottery workers who were exposed to the lead hazard to certain precautionary measures designed to reduce this hazard involved in pottery production.